

### REMARKS

Please enter this Second Preliminary Amendment.

A review of the initial Preliminary Amendment revealed a few clerical matters, which would be appropriate to correct before examination on the merits.

An amendment was twice proposed for page 5, beginning at page line 10 through page 6, ending at line 17. The second such proposed amendment (Preliminary Amendment at page 2 to page 3) should be presented with respect to page 8, beginning at line 21 through page 9, ending at line 15. The corrected amendment is enclosed herewith.

The amendments to the specification at page 6, line 19 to page 7, line 17 conform nomenclature.

The amendment to page 14, paragraph starting at line 15, inserts conventional Greek symbols and conforms the temperature to conventional U.S. format.

The amendment to page 15, paragraph starting at line 8, conforms the temperature to conventional format.

Applicants reserve the right to file a substitute specification with the aforesaid amendments for publication.

Respectfully submitted,

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**APPENDIX**  
**AMENDMENTS TO THE SPECIFICATION**

Amendments to the paragraph at page 6, beginning at line 19, through page 7, ending at line 17:

Fig. 6 is a pair of photographs of electrophoresis of PCR products for NB-1 and MASS-NB-SCH-1 genomic DNA using primers designed based on PAC clone data. The gel used was 2.5 agarose. (Lane 1: marker (1202 bp, 517 bp, 396 bp, 201 bp), Lane 2: positive control (human placenta DNA), Lane 3: normal cells derived from neuroblastoma patient without 1p36 deletion, Lane 4: tumor cells derived from neuroblastoma patient without 1p36 deletion, Lane 5: normal cells derived from MASS-NB-SCH-1 patient, Lane 6: MASS-NB-SCH-1, Lane 7: NB1, Lane 8: positive control (mouse hybridoma with human chromosome), Lane 9: positive control (human lymphocytes), Lane 10: positive control (human placenta DNA), Lane 11: normal cells derived from neuroblastoma patient without 1p36 deletion, Lane 12: tumor cells derived from neuroblastoma patient without 1p36 deletion, Lane 13: normal cells derived from MASS-NB-SCH-1 patient, Lane 14: MASS-NB-SCH-1, Lane 15: NB1, Lane 16: positive control (mouse hybridoma with human chromosome), Lane 17: positive control (human lymphocytes), Lane 18: empty lane). In A, dJ1028013-SP6[-spb] was used as primer for Lanes 1-8; dJ142A6-T7 was used as primer for Lanes 9-17. In B, dJ371E1-SP6 was used as primer for Lanes 1-8; dJ587c9-SP6 was used as primer for Lanes 9-17.

Amendments to the paragraph at page 8, beginning at line 21, through page 9, ending at line 15:

Identification of the tumor suppressor gene is possible by analysis of Loss of Heterozygosity (hereunder also abbreviated as LOH). The chromosomes of human cells, except for the sex chromosomes, exist as pairs, and two copies of each gene are present, one from the mother and one from the father. According to the "two-hit theory" advocated by Kundson A.G. et al. (Kundson A.G., *Pediatr. Res.*, 10:513- (1976)), inactivation of a gene is only found to occur when both of each copy from the mother and father are inactivated, and LOH is observed most

often by this mechanism. LOH refers to a pattern whereby a small genetic abnormality (deletion or substitution of a base or part of the gene) exists in one copy, while the chromosome on which the other copy resides undergoes the entire deletion of a large region including the gene.

Consequently, in patients experiencing onset of cancer due to inactivation of a tumor suppressor gene, it is thought that there are two copies of the chromosome on which the tumor suppressor gene resides in normal cells, and one copy in tumor cells.

Amendments to the paragraph at page 14, beginning at line 15:

Two of the obtained confluent culturing plates were used to obtain cells by trypsin treatment. The obtained cells were suspended in 5 ml of TEN buffer (TEN: 50 mM Tris-Cl (pH 8.0), 1 mM EDTA, 100 mM NaCl) and homogenized. To the homogenized suspension there were added 750  $\mu$ l [1] of SDS (10%) and 125  $\mu$ l [1] of Proteinase K (MERCK Co., 20 mg/ml), and the mixture was gently mixed by inversion and incubated overnight at [50-C] 50°C for lysis of the cells.

Amendments to the paragraph at page 15, beginning at line 8:

Next, 10.2 ml of ethanol which had been precooled at [-20-C] -20°C was added to and mixed with the collected supernatant, the resulting filamentous DNA was collected with a Pasteur pipette, the excess ethanol was removed and the DNA was dried. An appropriate amount of TE buffer (Tris-Cl (pH 8.0), 1 mM EDTA) was added to the dried DNA, and mixing was effected at room temperature for 1-2 days to dissolve the DNA.

(Example 2) Purification of genomic DNA extracted from human neuroblastoma cell lines.